RESEARCH PAPER

Variation in heterostylous breeding systems in neighbouring populations of *Oxalis alpina* (Oxalidaceae)

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ABSTRACT

The heterostylous reproductive system of *Oxalis alpina* in the Galiuro Mts. of Arizona was investigated using field surveys, controlled crosses in the greenhouse and measurements of reproductive morphs. Although populations in the Pinaleño Mts. to the immediate east and in the Santa Catalina Mts. to the immediate west have derived distylous reproductive systems, tristyly, the ancestral reproductive system in *O. alpina*, has been retained in the Galiuro Mts. population. Tristylous incompatibility relationships in the Galiuro population are modified from the ancestral condition, with significant loss of incompatibility differentiation between stamen whorls of both short- and long-styled morphs. Morphological adjustments of anther positions in the Galiuro population of *O. alpina* match those expected in light of incompatibility modification, with divergence of the mid-level anthers away from the position of the mid stigmas of the mid-styled morph. The occurrence of tristyly in an area of Arizona where distyly is found in adjacent mountain ranges is particularly remarkable, and indicates both the isolation of populations restricted to the upper elevations of these mountain ranges and variation in the tempo of evolution over short geographic distances.

INTRODUCTION

The Sky Islands of the Sonoran Desert, well known for their high biotic diversity, provide an outstanding opportunity for evolutionary studies. These 'islands' are the tops of mountain ranges surrounded by desert, and represent the northernmost extent of the Sierra Madre Occidental in northern Mexico. The Sky Islands are located in southeastern Arizona and southwestern New Mexico in the United States, and Sonora and Chihuahua in Mexico. Their high biotic diversity probably results from isolation of the Sky Island mountain ranges and climatic fluctuations since the end of the Pleistocene (Weller 1978, 1979; Van Devender & Spaulding 1979; Van Devender 1990; Masta 2000; Boyd 2002; McCormack *et al.* 2008; Tennessen & Zamudio 2008; Mitchell & Ober 2013).

We compared the reproductive system of *Oxalis alpina* in the Galiuro Mts. to other populations of this species located throughout the Sky Islands. Differentiation among populations of the same species occurring in these isolated mountain ranges may be pronounced, as exemplified by the variation in heterostylous reproductive systems of *O. alpina* populations in this region (Weller *et al.* 2007; Pérez-Alquicira *et al.* 2010; Sosenski *et al.* 2010; Weber *et al.* 2013a). In Sky Island populations of *O. alpina*, populations are either distylous, where two style morphs occur in populations (short- and long-styled morphs; Fig 1A) or tristylous (Fig. 1B), where three floral morphs occur in populations (short-, mid- and long-styled morphs). In tristylous populations, stigmas are located at either the short,

mid or long positions. Anther whorls are located at positions corresponding to the levels of the stigmas of the remaining two floral morphs. In distylous populations, stigmas are located at either the short or long positions, with anther whorls located at the level corresponding to the position of the stigmas in the opposite floral morph. The mid-styled morph is missing in distylous populations.

In addition to the striking difference in number of floral morphs of tristylous *versus* distylous populations of *O. alpina* in the Sky Islands, many less obvious differences occur among the tristylous populations, including modifications of tristylous incompatibility and floral morphology. These modifications suggest that distyly is the derived condition in *O. alpina*. In most heterostylous species, pollinations occurring between anthers and stigmas at the same level lead to fruit and seed production. These crosses were termed legitimate by Darwin (1877). Illegitimate crosses, which typically produce few seeds, include all outcrosses between different levels, as well as self-pollinations. Some Sky Island populations of *O. alpina* possess typical tristylous incompatibility relationships, where only crosses between anthers and stigmas at the same level produce seed. In many populations, however, two illegitimate crosses produce seed (the S × mL and L × mS crosses; see Fig. 1 for notation), and in some cases produce as many seeds as the corresponding legitimate S × sL and L × lS crosses (Fig. 1C). As seed production of these two illegitimate crosses increases, the short- and long-styled morphs become completely reciprocally intercompatible (all crosses between short- and long-styled
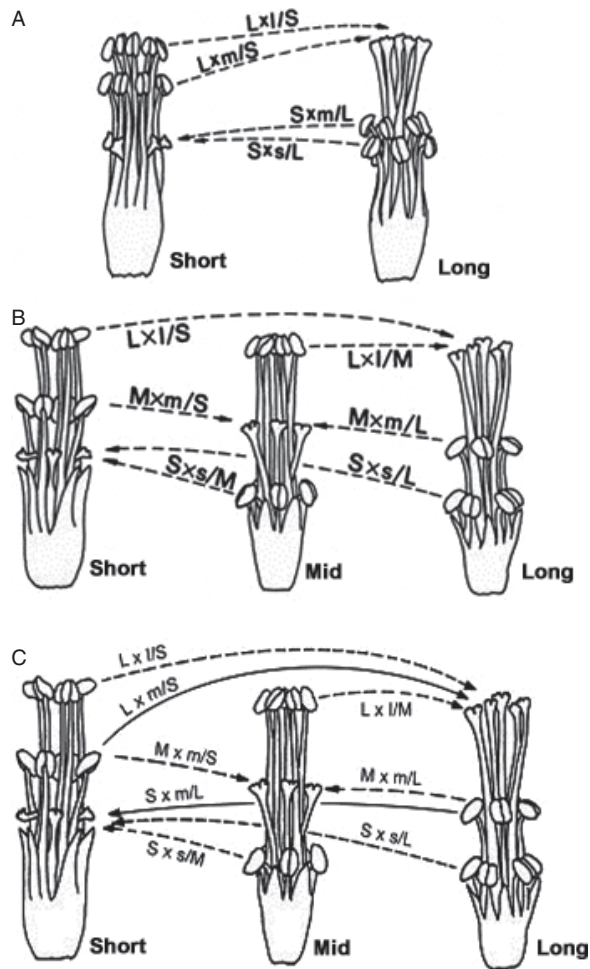
Fig. 1. Legitimate and illegitimate crosses in tristylous and distylous populations of *O. alpina*. As an example of cross notation, L × lS refers to a cross in which L represents the long-styled morph used as a female parent and pollinated with pollen from the long anther whorl of a short-styled morph. A. Legitimate crosses in a distylous population of *O. alpina*. Arrows show crosses leading to fertilisation of ovules and seed production. B. Legitimate crosses in a tristylous population of *O. alpina* with unmodified incompatibility. Only those crosses occurring between anthers and stigmas at the same level lead to fertilisation of ovules and seed production. C. Crosses that lead to fertilisation of ovules and seed production in a population of *O. alpina* with modified tristylous incompatibility. The S × mL and L × mS crosses may produce fewer seeds that their legitimate counterparts (the S × sL and L × lS crosses, respectively), in which case tristylous incompatibility is partially modified, or the same number of seeds as the legitimate crosses (fully modified tristylous incompatibility). The S × mL and L × mS crosses are considered illegitimate in tristylous populations, but legitimate in distylous populations.

morphs produce seeds), and selection favours genes carried by the short- and long-styled morphs, rather than genes carried in the outnumbered pollen of mid-styled morphs (Weller *et al.* 2007; Weber *et al.* 2013a). These incompatibility modifications select against the alleles resulting in expression of the mid-styled morph (M-alleles; Weller *et al.* 2007), which are carried primarily by pollen from the mid-styled morph. Decrease in frequency of M-alleles eventually results in decreased frequency of the mid-styled morph.

Increased self-compatibility of the mid-styled morph (relative to short- and long-styled morphs), in addition to modifications of tristylous incompatibility, is expected to result in expression of inbreeding depression in the progeny of mid-styled morphs, and lead to reduced frequency of this floral morph relative to short- and long-styled morphs (Charlesworth 1979). Empirical studies support this prediction; mid-styled morphs have higher self-compatibility, self-fertilise more, and express more inbreeding depression in their progeny than short- and long-styled morphs (Weber *et al.* 2013a).

Substantial climate oscillation since the last glacial maximum (Van Devender & Spaulding 1979; Van Devender 1990) may have played an additional role in the evolution of distyly. Reduction in population size may enhance the likelihood of loss of mid-styled morphs already rare due to modifications of tristylous incompatibility and increased self-compatibility of mid-styled morphs (Pérez-Alquicira *et al.* 2010). Fluctuations in population size in the northwestern Sky Islands, which are at the limits of the distribution of *O. alpina*, may have been particularly pronounced and caused decreased genetic variation and loss of the mid-styled morph in this region.

We investigated the heterostylous reproductive system of *O. alpina* in the Galiuro Mts. in southeastern Arizona. The Galiuro Mts. are located between the Santa Catalina Mts., 48 km to the west, and the Pinaleño Mts., 37 km to the northeast, which both have extensive and uniformly distylous populations (Fig. 2). These neighbouring distylous populations appear to be closely related to the Galiuro population, based on the occurrence of identical sequences in two regions of the chloroplast genome (Pérez-Alquicira *et al.* 2010). More distant populations of *O. alpina* to the north, in the Pinal and Sierra Ancha Mts., are also distylous. To the southeast in the Chiricahua Mts., the closest population of *O. alpina*, in Pinery Canyon, is also distylous, although tristylous populations occur further south in the Chiricahua Mts. (Fig. 2). Other tristylous populations occur at longer distances directly south in the Huachuca Mts., and to the northeast in the White Mts. Based on the prevalence of distyly in mountain ranges adjacent to the Galiuro Mts., and the occurrence of only short- and long-styled individuals in a small population surveyed in 1977 at the north end of the Galiuro Mts., populations in this mountain range were assumed to be distylous (Weller *et al.* 2007). Because the Galiuro Mts. have only a few areas of high elevation and consequently little coniferous forest compared to many of the mountain ranges in the Sky Islands, *O. alpina* is uncommon, complicating assessment of the reproductive system. To better characterise heterostyly in *O. alpina* from the Galiuro Mts., we carried out more extensive field observations, a greenhouse crossing programme to assess details of the incompatibility system and measurements of anther and stigma positions. We expected to find distyly in Galiuro populations of *O. alpina* based on our previous information, the prevalence of distyly in neighbouring mountain ranges, and the identical chloroplast sequences in these populations.

MATERIAL AND METHODS

Study species and style morph representation

Oxalis alpina (Rose) Knuth (section *Ionoxalis*) occurs from Guatemala to the southwestern United States (Denton
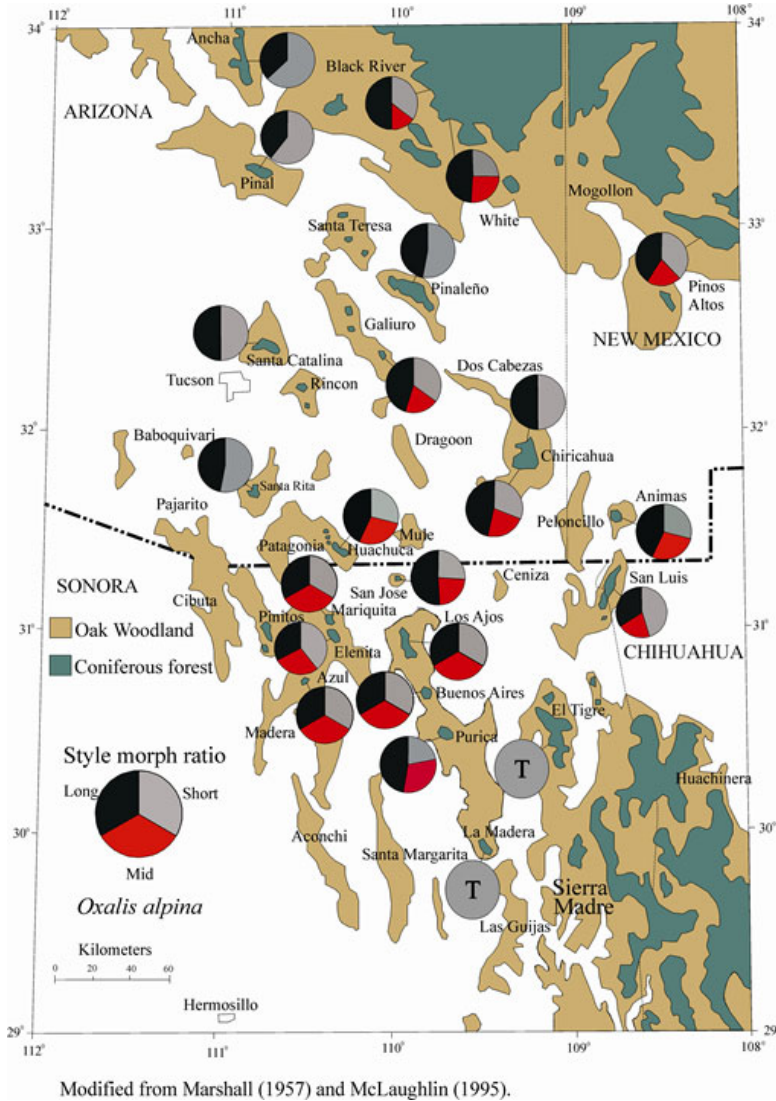
Fig. 2. Location of the tristylous Galiuro Mts. population of *O. alpina* relative to other Sky Island populations in the Sonoran Desert region. Distylous populations occur 48 km to the west in the adjacent Santa Catalina Mts., and 37 km to the east in the adjacent Pinaleño Mts. Frequencies of short-, mid- and long-styled morphs are shown in the pie diagrams for each population. For several populations, limited field observations or herbarium records were sufficient to indicate the presence of tristyly (T), but not the ratios of the style morphs. The distribution of oak woodland and coniferous forest follows Marshall (1957) and McLaughlin (1995), with modifications based on additional field observations (Weller *et al.* 2007).

Modified from Marshall (1957) and McLaughlin (1995).

1973), although recent phylogenetic studies (Gardner *et al.* 2012) show that this species, as currently defined, is polyphyletic. Populations in the Sky Islands, however, are likely to be monophyletic, based on possession of similar genetic markers (Pérez-Alquicira *et al.* 2010) and uniform tetraploidy in the populations which have chromosome counts (Weller & Denton 1976). We visited the Galiuro Mts. in August of 2007 and 2008. Bulbs of flowering and vegetative plants were collected in 2007, and flowering plants only in 2008. Style morph ratios were assessed using the much larger 2008 survey of flowering plants; style morph data were collected from plants located at distances > 1 m from each other to minimise sampling the same genet. Analysis of microsatellite variation (Tsyusko, personal communication) has verified the absence of clonal growth in *O. alpina* at this sampling scale. Plants were sampled near the intersection of the Bassett Peak and Ash Creek Trails (32°30.919′N, 110°15.835′W, 658 m a.s.l.; Weller and Sakai 977, 978, US). We used a single population from the Galiuro Mts. because *O. alpina* is rare at the low elevations characteristic of this mountain range.

Incompatibility relationships

To assess the nature of the incompatibility system, we used at least ten individuals of each style morph as seed parents in crosses, and an equal or larger number of plants as male parents. Because three style morphs were found in the Galiuro population, indicating the occurrence of tristyly, we carried out six types of legitimate crosses, 12 types of illegitimate crosses, and six types of illegitimate self-pollinations. Pollinations were carried out in the greenhouse, with fine forceps dipped in alcohol between each pollination. At least three of the five stigmas were pollinated on each flower to ensure full seed set, and the stigmas were inspected visually to verify that they were coated with pollen. For each maternal parent, at least five crosses in each legitimate category were carried out, and two crosses in the remaining illegitimate categories, with the exception of the illegitimate S × mL and L × mS illegitimate crosses, where at least five crosses per maternal genotype were completed (these latter two categories often produce seeds in populations with modified tristylous incompatibility). For each type of outcross, several male parents were used. Including legitimate and illegit-

imate crosses and self-pollinations, 976 crosses were completed for the Galiuro population. The crossing categories for the Galiuro plants were identical to those carried out for other Sky Islands populations of *O. alpina* (Weller *et al.* 2007). Fruits were collected 14–18 days after pollination, when seeds were ripe but before explosive dehiscence of the fruits. For each maternal genotype, the average number of seeds per fruit was used as a measure of incompatibility following a particular cross. Unpollinated tagged flowers (*ca.* two flowers per genotype), serving as controls, produced no fruits (n = 76 tagged flowers), indicating that no potential pollinating insects occurred in the greenhouse.

Pre-planned comparisons (SAS Institute, 2002-2005; SAS Institute, Cary, NC, USA) were used to test whether seed production of the illegitimate S × mL and L × mS crosses was low relative to the legitimate S × sM, S × sL, L × lM and L × lS crosses, as expected in typical tristylous incompatibility, or similar in seed production to these legitimate crosses, indicating loss of a key feature of typical tristylous incompatibility. Average values for seed production by maternal genotype and cross type were used in analyses to avoid pseudoreplication. Seed production from the illegitimate S × mL and L × mS crosses was also compared to remaining illegitimate crosses. A significant increase in seed production for the S × mL and L × mS crosses, relative to the remaining illegitimate crosses, indicates partial modification of tristylous incompatibility, even if seed production was less than for legitimate crosses. Using these comparisons, tristylous incompatibility in *O. alpina* from the Galiuro Mts. was categorised as fully modified (incompatibility relationships of short- and long-styled morphs identical to those found in distylous populations), partially modified (seed production of S × mL and L × mS crosses higher than for other illegitimate crosses, but less than seed production of legitimate crosses), or unmodified (seed production of S × mL and L × mS crosses identical to other illegitimate crosses; Weller *et al.* 2007). To compare the extent of incompatibility modification of the Galiuro population with other populations of *O. alpina*, the mean number of seeds per cross from the illegitimate S × mL cross was divided by the mean number of seeds per cross for the legitimate S × sL cross. Similarly, the mean number of seeds per cross for the L × mS cross was divided by the mean number of seeds per cross for the L × lS cross. These ratios for short- and long-styled morphs were averaged, with a higher value indicating greater modification of tristylous incompatibility. A value of 1 indicates complete loss of incompatibility differentiation between the stamen whorls within short-styled flowers and within long-styled flowers, and an incompatibility system indistinguishable from distyly, considering only the short- and long-styled morphs.

Seed production following self-pollination was used to evaluate the potential for expression of inbreeding depression. Previous studies have indicated considerable self-compatibility in mid-styled morphs in populations when incompatibility relationships in the short- and long-styled morphs are modified (Weller *et al.* 2007; Weber *et al.* 2013a).

Morphological variation of floral morphs

As the mid morph becomes rare in populations as a result of incompatibility modifications and expression of inbreeding depression, mid-level anther positions of short- and long-styled

floral morphs are expected to converge toward the position of the styles of the long- and short-styled floral morphs, respectively. To test this prediction for plants from the Galiuro population, floral measurements were made for 30 short- and 19 long-styled floral morphs, using protocols established by Sosenski *et al.* (2010). For each genet, the lengths of the two stamen whorls and the stigmas were measured for five individual flowers. These values were averaged for each genet, and the values of genets averaged to provide mean values across all individuals of each style morph. Within-morph reciprocity values ($R_{W_{Short}}$ and $R_{W_{Long}}$) indicate the degree of convergence of two anther whorls within short- and long-styled morphs, respectively, as

$$R_{W_{Short}} = 1 - \frac{|mS - lS|}{L} \text{ and } R_{W_{Long}} = 1 - \frac{|mL - sL|}{L} \quad (1)$$

where lS and mS refer to anther positions for long and mid stamens of the short-styled morph, respectively, mL and sL refer to anther positions for mid and short stamens of the long-styled morphs, respectively, and *L* refers to the length of the long stigmas of the long-styled morph. Between-morph reciprocity values (($R_{B_{Short}}$ and $R_{B_{Long}}$) indicate the degree of convergence between mid-anther whorls and stigmas of long- and short-styled morphs, respectively, as

$$R_{B_{Short}} = 1 - \frac{|mS - L|}{L} \text{ and } R_{B_{Long}} = 1 - \frac{|mL - S|}{L} \quad (2)$$

The reciprocity index (R_I) is the average of $R_{W_{Short}}$, $R_{W_{Long}}$, $R_{B_{Short}}$ and $R_{B_{Long}}$ (Sosenski *et al.* 2010; note that in Sosenski *et al.* (2010) the absolute value signs for mS-lS and mS-L were inadvertently left out of the formulae, P. Sosenski, personal communication (2015)).

Comparisons among populations of *O. alpina*

Style-morph frequency, incompatibility modification and the extent of morphological modification in the Galiuro population were compared to other populations of *O. alpina* throughout the Sky Islands, using data from Weller *et al.* (2007) and Sosenski *et al.* (2010). The populations used in analyses varied slightly for different regression analyses, depending on the availability of data for each population.

RESULTS

Style-morph representation

Oxalis alpina in the Galiuro Mts. is tristylous, with 18 short-styled morphs (35.3%), ten mid-styled morphs (19.6%) and 23 long-styled morphs (45.1%), based on a sample of 51 flowering plants in 2008.

Incompatibility relationships

Following controlled crosses, seed production per cross of the illegitimate S × mL and L × mS crosses was significantly lower than for the legitimate S × sM, S × sL, L × lM and L × lS crosses using pre-planned comparisons (F = 14.33, df = 1, P = 0.0003; Fig. 3). The illegitimate S × mL and L × mS

crosses had higher seed production than the remaining illegitimate crosses (F = 143.87, df = 1, P = 0.0001; Fig. 3). Together, these data indicate that the Galiuro population has partial loss of incompatibility differentiation (Weller *et al.* 2007). The value indicating the extent of loss of incompatibility differentiation in the Galiuro population was 0.63.

The six legitimate crosses differed significantly in seed production (F = 5.98, df = 5, P = 0.0001, largely because of reduced seed production of the M × mS cross (P < 0.05, Tukey's *post-hoc* comparison; Fig. 3). Seed production following self-pollination differed significantly by cross, with highest seed production for the M × lM and M × sM self-pollinations (F = 4.32, P = 0.0019). A Tukey's *post-hoc* test indicated that the M × lM cross had higher seed production than the S × lS and L × sL self-pollinations (Fig. 3).

Morphological variation of floral morphs

Short- and long-styled morphs of *O. alpina* in the Galiuro population showed considerable morphological modification compared to typical tristylous species. Short-styled morphs from the Galiuro population had higher values for within- and between-morph reciprocity ($R_{W_{Short}}$ = 0.827; F = 3.34, df = 1, P = 0.098 and $R_{B_{Short}}$ = 0.851; F = 7.55, df = 1, P = 0.021) than for short-styled morphs in other tristylous Sky Island populations. In contrast, long-styled morphs from the Galiuro population had reciprocity values similar to those of other populations in the Sky Islands (R_{Wlong} = 0.799; F = 0.01, df = 1, P = 0.935 and $R_{B_{Long}}$ = 0.806, F = 0.01, df = 1, P = 0.941). The overall reciprocity index (R_i) was 0.821 for the Galiuro population, higher than the corresponding values for other tristylous populations, suggesting a closer similarity than expected to the morphological features of distylous populations.

Comparisons among populations

The regression of the mid-styled morph frequency on the extent of incompatibility modification was significant with a negative slope when incompatibility data from the Galiuro Mts. were included with values for other populations (F = 10.29, df = 1, 10, P = 0.0107; regression $y = -13.2x + 35.1$; Fig. 4). A signifi-

cant negative slope is expected if incompatibility modification has a causal role in the decline in frequency of mid-styled morphs. A regression of the reciprocity index (R_i) against the extent of incompatibility modification was significant, with a positive slope (F = 14.13, df = 1,9, P = 0.0045; regression $y = 0.101x + 0.706$; Fig. 5), suggesting that as increasing modification of tristylous incompatibility causes a decline in the frequency of the mid-styled morph, adjustments in the positions of mid-level anthers occur that lead to higher pollen transfer from these anthers to stigmas of short- and long-styled morphs.

DISCUSSION

Evidence from crossing studies among populations (Weller 1978) and phylogeographic studies based on chloroplast sequences (Pérez-Alquicira *et al.* 2010) indicate that distyly has evolved independently on several occasions within the Sky Islands distribution of *O. alpina*. The retention of tristyly at the southern end of the Galiuro Mts., an area surrounded to the west, north and east by mountain ranges with uniformly distylous populations, is consistent with the occurrence of these transitions at different times. Based on spatial analysis of molecular variance and UPGMA using variation at two chloroplast DNA markers (Pérez-Alquicira *et al.* 2010), the tristylous Galiuro population clustered with the Pinos Altos (tristylous), Pinaleño (distylous), Chiricahua (distylous Pinery population), White (tristylous), Santa Catalina (distylous) and San Luis (tristylous) populations. The mix of tristylous and distylous populations within this genetically distinct group suggests that distyly has evolved independently and perhaps on several occasions within the northwestern Sky Islands (Pérez-Alquicira *et al.* 2010). The same pattern of co-occurrence of tristyly and distyly is found in a second phylogeographically distinct group including the Chiricahua (Morse Canyon; tristylous), Santa Rita (distylous) and Huachuca (tristylous) populations. Distyly has also evolved independently in a third, more distantly related, group consisting of populations from the Pinal and Sierra Ancha populations (Pérez-Alquicira *et al.* 2010). The independent evolution of distyly within the Sky Islands is further supported by the increased genetic differentiation among distylous compared to tristylous populations (Pérez-Alquicira *et al.* 2010). The absence of genetic differentiation with the two groups containing tristylous and distylous populations suggests that shifts to distyly have been recent.

Populations of *O. alpina* have probably been isolated since the end of the Pleistocene (Van Devender & Spaulding 1979; Van Devender 1990), and there is no evidence for recent gene flow among mountain ranges or among populations within the same mountain range. Unique chloroplast haplotypes occur in different mountain ranges, indicating a lack of gene flow *via* seeds between populations (Pérez-Alquicira *et al.* 2010), and there is no evidence for exchange of the M-allele (a nuclear marker) between populations *via* pollen (Weller 1986). Seeds are ballistically dispersed, but over short distances. Pollinators of *O. alpina* are small native bees (Baena-Díaz *et al.* 2012) that probably fly over short distances, and would be unlikely to facilitate gene flow among isolated populations. Even within the Chiricahua Mts. where both tristylous and distylous populations occur, gene flow appears to be limited, as there is no evidence for movement of M-alleles from tristylous to distylous populations (Weller 1986).
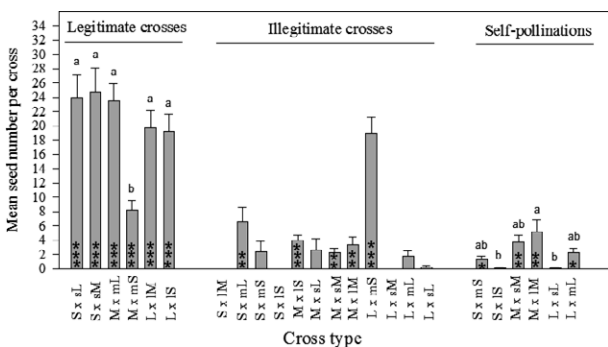
Fig. 3. Seed production following legitimate, illegitimate and self-pollinations of *O. alpina* from the Galiuro Mts. For each cross, mean values are based on averages by maternal parent. Bars represent SE. Asterisks indicate that values of seed production differ significantly from zero (*P < 0.05, **P < 0.01, ***P < 0.001). Bars with identical lowercase letters indicate either legitimate pollinations or self-pollinations that did not differ significantly in seed production within each category of crosses.
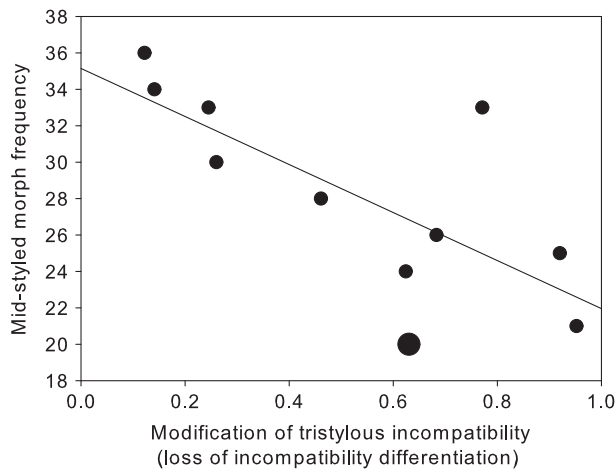
Fig. 4. Regression of mid-styled morph frequency against the extent of loss of incompatibility differentiation in 11 populations of *O. alpina* sampled for morph frequency in the field. The Galiuro population is indicated by the larger symbol. Higher values on the *x*-axis indicate tristylous incompatibility more closely resembling typical distylous populations, where the S × mL and S × sL crosses as well as the L × mS and L × lS crosses have equivalent seed production. Regression equation is $y = -13.2x + 35.1$.
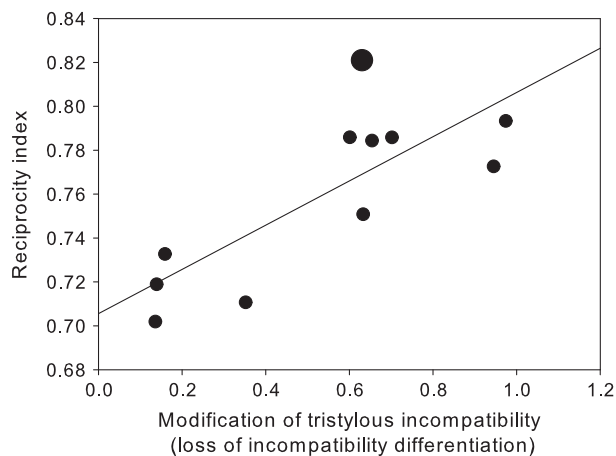
Fig. 5. Regression of reciprocity (R_I) against incompatibility modification for 11 populations of *O. alpina*. Reciprocity is calculated as the average of with-morph and between-morph convergence for short- and long-styled morphs. See text for explanation. The Galiuro population is indicated by the larger symbol. Higher values on the *x*-axis indicate tristylous incompatibility more closely resembling typical distylous populations, where the S × mL and S × sL crosses as well as the L × mS and L × lS crosses have equivalent seed production. Regression equation is $y = 0.101x + 0.706$.

Close genetic relationships similar to those of tristylous *O. alpina* in the Galiuro Mts. and neighbouring distylous populations in the Santa Catalina and Piñaleno Mts. have been found in two other unrelated taxa (tree frogs; Barber 1999; and some populations of jumping spiders; Masta 2000), although for a beetle (*Scaphinotus petersi*), the Santa Catalina and Piñaleno populations were placed in two different major clades (Mitchell & Ober 2013; beetles from the Galiuro Mts. were not included in this study). Although fluctuations in climate since the last glacial maximum are often used to explain biogeographic patterns in the Sky Islands, some studies indicate a mismatch between patterns of genotypic divergence

and a causal explanation based on these climate fluctuations (Tennessen & Zamudio 2008). No obvious reason is apparent for the retention of tristyly in the Galiuro Mts. and the evolution of distyly in neighbouring mountain ranges. *Oxalis alpina* is scarce in the Galiuro Mts. due to the generally low elevation of this mountain range and absence of suitable high-elevation habitat. Small population size might generally favour loss of the mid-styled morph *via* stochastic events when mid-styled morphs are already uncommon due to selection. Stochastic events of this nature may explain the occurrence of distyly in two highly differentiated distylous populations in the extreme northwestern region of the Sky Islands (Pinal Mts. and the Sierra Ancha; Pérez-Alquicira *et al.* 2010). Mid-styled morph frequency appears to be reduced in the Galiuro Mts., consistent with the likelihood of stochastic events and loss of the mid-styled morph in small populations. If stochastic events are important, however, the retention of some mid-styled morphs in the Galiuro Mts., and loss of this morph in the much larger distylous populations of the neighbouring Santa Catalina and Pinaleño Mts., is puzzling. Additional style morph surveys of the northern part of the Galiuro Mts. would be of interest, as would similar surveys of *O. alpina* in the Santa Teresa Mts. immediately north of the Galiuro Mts.

As in other tristylous populations in the northern Sky Islands, Galiuro *O. alpina* has partial loss of incompatibility differentiation between anther whorls in both the short- and long-styled morphs (Fig. 1C). Modified tristylous incompatibility is expected to favour short- and long-styled morphs as pollen parents at the expense of pollen from mid-styled morphs, which carry most of the *M*-alleles (Weller *et al.* 2007). The prediction for loss of the mid-styled morph is supported by the negative slope for mid-styled morph frequency with increasing modification of tristylous incompatibility (Fig. 4). The low observed value for frequency of the mid-styled morph from the Galiuro Mts. relative to the value predicted by the regression may result from reduction in the frequency of this morph through stochastic events.

Additional modifications of the tristylous reproductive system in the Galiuro population are characteristic of those found in other populations with partially modified tristylous incompatibility. Mid-styled morphs show considerable self-compatibility, which is likely to result in increased self-fertilisation and the expression of inbreeding depression in their progeny, based on comparison to other *O. alpina* populations with similar patterns of self-compatibility (Weber *et al.* 2013a). Higher relative expression of inbreeding depression in mid-styled morphs compared to short- and long-styled morphs is a likely factor in loss of mid-styled morphs from populations with modified tristylous incompatibility (Charlesworth 1979; Weller *et al.* 2007; Weber *et al.* 2013a).

Seed production following the legitimate M × mS cross in Galiuro plants of *O. alpina* was reduced compared to other legitimate crosses, a pattern found throughout Sky Island populations of *O. alpina* with modified tristylous incompatibility (Weller *et al.* 2007). In other tristylous populations with this additional modification of incompatibility, fewer than expected short-styled morphs were found in the progeny of mid-styled morphs grown from field-collected seeds or from progeny grown following crosses in the greenhouse where legitimate pollen from short- and long-styled morphs was placed on mid

stigmas (Weber *et al.* 2013b). The unequal style morph representation following these two categories of legitimate crosses is the result of reduced pollen tube growth following M × mS crosses (Weber *et al.* 2013b). A consequence of the higher success of pollen from long-styled morphs on stigmas of mid styled morphs is increased representation of mid-styled morphs in the progeny, which may slow loss of the mid-styled morphs in the Galiuro Mts. and in other populations where modifications of tristylous incompatibility favour short- and long-styled morphs (Weller *et al.* 2007; Weber *et al.* 2013a).

Morphological modifications leading to higher reciprocity between short- and long-styled morphs were evident in the Galiuro population, as in other Sky Island populations with modified tristylous incompatibility. The increased convergence of stamens in the Galiuro population, relative to other populations with similar incompatibility modification, resulted primarily from the high value for between-morph reciprocity of the short-styled morph ($R_{B_{Short}}$), and to a lesser extent, the high value for within-morph convergence of stamens for the short-styled morph ($R_{W_{Short}}$). The high value for reciprocity (R_I) in the Galiuro Mts. population of *O. alpina* is characteristic of tristylous populations with completely modified incompatibility (*e.g.* Morse Canyon, Chiricahua Mts.) and distylous populations (Santa Catalina Mts.; Sosenski *et al.* 2010).

The reproductive system of *O. alpina* in the Galiuro Mts. is consistent with the hypothesis that breeding systems have evolved independently in Sky Island populations of this species.

The heterogeneous geographic distribution of distyly and tristyly, the substantial variation in tristylous incompatibility and morphology, and the occurrence of distinct groups based on chloroplast sequences all suggest differences in the pace of evolution, even over very short geographic distances. Imposed over this heterogeneity, however, is a general pattern of higher modification of tristyly in the northern Sky Islands, culminating in the numerous distylous populations in the northwestern portion of the region. Stochastic events associated with fluctuations in population size appear to magnify the effects of selection against mid-styled morphs (Pérez-Alquicira *et al.* 2010), and have led to the current distribution of breeding systems in this region.

ACKNOWLEDGEMENTS

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